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Virus-Directed Enzyme Prodrug Therapy: Intratumoral Administration of a Replication-Deficient Adenovirus Encoding Nitroreductase to Patients With Resectable Liver Cancer

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ABSTRACT

Purpose
Virus-directed enzyme prodrug therapy depends on selective delivery of virus encoding a prodrug-activating enzyme to tumor, followed by systemic treatment with prodrug to achieve high levels of the activated cytotoxic at the intended site of action. The use of the bacterial enzyme nitroreductase to activate CB1954 (5-(aziridin-1-yl)-2,4-dinitrobenzamide) to a short lived, highly toxic DNA cross-linking agent has been demonstrated in tumor xenografts. In this study, we report the first clinical trial investigating the feasibility, safety, and transgene expression of a replication-defective adenovirus encoding nitroreductase (CTL102) in patients with liver tumors.

Patients and Methods
Patients with resectable primary or secondary (colorectal) liver cancer received a single dose of CTL102 delivered by direct intratumoral inoculation 3 to 8 days before surgical resection.

Results
Eighteen patients were treated with escalating doses of CTL102 (range, 10^8–5×10^{11} virus particles). The vector was well tolerated with minimal side effects, had a short half-life in the circulation, and stimulated a robust antibody response. Dose-related increases in tumoral nitroreductase expression measured by immunohistochemical analysis have been observed.

Conclusion
Direct intratumoral inoculation of CTL102 to patients with primary and secondary liver cancer is feasible and well tolerated. The high level of nitroreductase expression observed at 1 to 5×10^{11} virus particles mandates further studies in patients with inoperable tumors who will receive CTL102 and CB1954.

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INTRODUCTION

Although many conventional cytotoxic drugs can achieve 100% tumor-cell kill in vitro, their efficacy in vivo is constrained by dose-limiting systemic toxicity. Virus-directed enzyme prodrug therapy (VDEPT) aims to overcome this limitation by utilizing a genetically modified virus to express an enzyme that converts an inactive prodrug to a cytotoxic metabolite in infected cells. Provided the virus can be delivered selectively to the tumor and the activated cytotoxic species has a short half-life, this will maximize local tumor-cell kill while minimizing systemic toxicity. Tumor selectivity can be conferred through selective delivery to cancer sites, cancer specific promoters, or retargeted viruses with altered tropism.

Many gene therapy strategies, such as tumor suppressor gene correction, are limited by the inability of current vectors to transduce more than a small proportion of the target tumor cell population to express the therapeutic gene. An important facet of the VDEPT approach is that the short-lived

toxic metabolite is able to diffuse into and kill surrounding nontransduced cells, so that even when only a relatively small proportion of target cells are transduced, significant cell killing can still occur (bystander effect).

CB1954 (5-(aziridin-1-yl)-2,4-dinitrobenzamide) stimulated interest when it was found to be highly active against Walker rat 256 carcinoma cells;[1] conversion from a weak mono-functional alkylating agent to a highly potent bifunctional cytotoxic (100,000 times more potent than the prodrug) was effected by endogenous rat DT-diaphorase (EC1.6.99.2).[2] However, CB1954 is a poor substrate for human DT-diaphorase, explaining the poor activity against human tumors.[3] Subsequent studies demonstrated that the enzyme nitroreductase (NTR; EC1.6.99.7), encoded by the *nfsB* gene of *Escherichia coli* B, can perform this bioreduction 100-fold more efficiently than rat DT-diaphorase.[4]

These properties make the combination of CB1954 and NTR exploitable by a VDEPT approach. To this end, we have constructed CTL102, an E1, E3-deleted replication-deficient human adenovirus serotype 5 vector, engineered to contain the *E coli nfsB* gene under the control of the cytomegalovirus immediate early (CMV IE) promoter.[5,6] Preclinical studies have shown that adenoviral delivery of NTR to a range of human cancer cell lines sensitizes them to CB1954 by 500- to 2,000-fold compared to the parental cell line.[5] Administration of CTL102 and CB1954 has given strong antitumor effects in many in vivo mouse xenograft models, including a doubling in median survival in a peritoneal model of pancreatic cancer[5] and complete cures in a subcutaneous model of primary liver cancer.[6]

In vitro cell mixing experiments using unmodified and NTR-expressing ovarian carcinoma cell lines have demonstrated significant sensitization (30- to 100-fold) of the total cell population to CB1954 when only 5% to 10% of the cells express NTR.[7] Similarly, in vivo in a human hepatoma murine xenograft model, a significant antitumor effect and improved survival was observed even when only 5% of cells expressed NTR, confirming a significant bystander effect.[8]

In contrast to the widely used thymidine kinase-ganciclovir system, where the activated drug requires gap junction communication or uptake of apoptotic cell fragments to reach neighboring cells,[9] activated CB1954 enters adjacent cells via a gap junction independent mechanism.[10,11] Further potential advantages of the NTR/CB1954 combination are the ability to kill cells in a cell-cycle independent manner, and the lack of cross-resistance with other commonly used cytotoxic agents.[5,7] Conversion of CB1954 to the active species relies on an intracellular cofactor (nicotinamide/adenine dinucleotide or nicotinamide/adenine dinucleotide phosphate), further increasing safety, since no extracellular activation can occur.

VDEPT has three potential sources of toxicity: vector, prodrug, and the vector/prodrug combination. We have already completed a phase I and pharmacokinetic study of the prodrug CB1954 in cancer patients.[12] Dose-limiting toxicity consisted of mild asymptomatic transaminitis and diarrhea. A dose of 24 mg/m^2 could be administered intravenously (IV) without significant side effects. The mean peak serum concentration of CB1954 following IV administration at this dose was 6.3 μmol/L, remaining above 1 μmol/L for 2 hours, thereby giving an area under the concentration-time curve of 5.8 μmol/L/h.[12] In vitro experiments using NTR-expressing cells have shown IC50 values for CB1954 in the range 0.1 to 5 μmol/L, suggesting that with adequate NTR expression, a therapeutic effect would be anticipated.[5,7,8]

Murine toxicology studies in nude and immunocompetent CD-1 mice have shown that an IV dose of 3×10^{11} virus particles was associated with a significant reduction in body weight and elevated liver transaminases. However, direct intratumoral injection of the same dose was confirmed to be a safe and reliable way of achieving tumor-specific transgene expression, with only a very low level of NTR expression detectable in normal liver.[7,8]

This article describes the first clinical experience of adenovirus encoding NTR, administered by direct intratumoral inoculation of increasing doses to patients with primary or secondary liver cancer awaiting hepatic resection. The primary clinical end point of the trial was toxicity. Secondary end points included the degree of expression of NTR in the tumor specimen, virus pharmacokinetics, and immune responses.

PATIENTS AND METHODS

Trial Design

This is a phase I dose escalation study of the replication-deficient adenovirus vector CTL102 encoding *E coli* NTR in patients with primary or secondary liver cancer undergoing surgical resection. The primary end point is to establish the safety and tolerability of the vector. Secondary end points are to assess efficiency of transgene expression, virus distribution, and antivector immune responses.

A minimum of three patients per dose level were treated, with expansion to a maximum of six in the event of toxicity. Dose-limiting toxicity was defined using the National Cancer Institute Common Toxicity Criteria as grade 2 renal, hepatic, or neurotoxicity, grade 3 mucositis or diarrhea, or grade 4 hematologic toxicity lasting more than 1 week or associated with fever, in at least two of six patients treated at that dose level. The maximum dose was restricted by current virus availability.

Patient Selection

Patients eligible for this study were those with hepatic metastatic colorectal cancer or with hepatocellular carcinoma 1 to 5 cm in diameter, accessible to ultrasound-guided injection, confined to one lobe of the liver, with no evidence of extra-hepatic disease, such that surgical resection could be undertaken. Other inclusion criteria were: life expectancy > 3 months; age > 18 years; WHO performance status 0 to 1; adequate hepatic, renal, and bone marrow function; normal blood clotting; and no potential

immuno-incompetence (including chemotherapy or radiotherapy within 4 weeks, concurrent corticosteroid use, or known HIV positivity). This study protocol was approved by the UK Gene Therapy Advisory Committee and the Local Research Ethics Committee (United Kingdom), and by the Central Committee on Research Involving Human Subjects, the Ministry of Housing, Spatial Planning and the Environment, and local ethical committees (the Netherlands).

Before treatment, in view of the theoretical risk of recombination between wild-type adenovirus and CTL102, samples (plasma, throat swab, urine, stool) were tested for adenovirus antigens. The presence of a concurrent wild-type adenovirus infection would exclude the patient from the study. Following treatment, patients were assessed weekly for 4 weeks, then monthly for 3 months, 3-monthly up to 1 year, and annually thereafter.

CTL102 Construction and Manufacture

The details of CTL102 construction have been described previously.[6] In brief, the *E coli B nfsB* coding sequence was cloned under control of the CMV IE promoter at position 345 in the E1-, E3-deleted Ad5 genome. CTL102 was manufactured in E1-expressing PER.C6 cells[13] by rescue of virus following transfection with two overlapping plasmids, together comprising the full CTL102 genome sequence. There is no homology between the E1 sequences in PER.C6 cells and the CTL102 vector, thus minimizing the generation of replication-competent adenovirus. Virus batches of 4.2×10^{10}–9.1×10^{11} particles/mL with particle:infectivity ratios in the range 15 to 20 were used. They were free of replication competent adenovirus at a detection limit of 1 in 10^9 plaque forming units (tested in A549 cells), meeting the specifications agreed with the UK regulatory authorities.

CTL102 Administration

CTL102 was administered by direct intratumoral injection in a volume of 0.25 mL of isotonic buffer (25 mmol/L Tris pH7.4, 0.14M NaCl, 5 mmol/L KCl, 0.6 mmol/L Na_2HPO_4, 0.9 mmol/L $CaCl_2$, 0.5 mmol/L $MgCl_2$, 5% sucrose) under ultrasound guidance using a 22-gauge needle into a single, preselected tumor deposit. The procedure was performed in a purpose-built gene therapy isolation suite and reverse barrier nursing was practiced until the absence of virus shedding was confirmed. This was followed 3 to 8 days later by laparotomy and hepatic resection. The initial dose was 1×10^8 virus particles/tumor. Dose escalation proceeded in 10-fold increments up to 1×10^{11} virus particles and subsequently in a five-fold increment to a maximum dose of 5×10^{11} virus particles.

Virus Shedding

Plasma, throat swab, urine, and stool were analyzed by enzyme-linked immunosorbent assay (ELISA; Dako IDEIA, Dako Ltd, Ely, UK) for the presence of adenovirus proteins before injection and at 24-hour intervals until virus clearance was confirmed. All samples were heat inactivated at 56°C for 30 minutes and assayed according to the manufacturer's protocol (100 μL aliquots assayed in triplicate). The detection limit was determined to be 2×10^5 particles CTL102.

Virus Kinetics

Peripheral venous blood samples were analyzed for the presence of vector DNA at the following times in relation to treatment: pretreatment, immediately on completion of virus injection, and 0.5, 1, 2, 4, 8, and 24 hours after injection. Taqman quantitative polymerase chain reaction (PCR) was performed on DNA extracted from 200 μL aliquots of whole blood using the QIamp DNA Mini Kit (Qiagen, Hilden, Germany) spin column method. Primers and probe specific for the CMV promoter-NTR junction were used to allow specific detection of CTL102 DNA, and quantitation was performed over 40 cycles of amplification using a standard curve of heat-inactivated CTL102. Total DNA recovery was monitored using an 18S rRNA gene primer and probe set (sequences provided by Ambion Inc, Austin, TX).

Immune Responses

Plasma samples were taken at pretreatment, days 7, 14, 21, and 28, and 2 and 3 months after treatment, heat-inactivated at 56°C for 30 minutes, and analyzed by ELISA to quantify total immunoglobulin response against adenovirus, NTR, and influenza A. For detection of antibodies to adenovirus, ELISA plates were coated with heat-inactivated Ad5 wild type virus at a concentration of 20 ng protein/well as previously described.[14] For NTR antibody ELISA, plates were coated with purified recombinant NTR[15] at 180 ng/well. In both assays, human antibodies (total immunoglobulin [Ig]) were detected with a horseradish peroxidase conjugated rabbit antihuman antibody and o-phenyldiamine substrate (Sigma, St Louis, MO). In the adenovirus ELISA assay, additional plates were tested for IgM, IgG, and IgA specific responses. Influenza A specific antibodies were assessed with a commercial ELISA kit (IBL, Hamburg, Germany). The neutralizing activity of patient plasma against Ad5 was tested using an E1, E3-deleted replication defective Ad5 virus encoding β-galactosidase under the CMV IE promoter in A549 cells.[14]

100 μL aliquots of heat-inactivated plasma dilutions were applied to 10^4 A549 cells in a 96 well plate and 100 μL virus dilution (2×10^5 particles/cell) added immediately afterwards. At 48 hours post infection, cell lysates were analyzed for β-galactosidase activity using a luminescence assay (Tropix, Bedford, MA) read on a Victor plate reader (Perkin Elmer Life Sciences, Cambridge, UK). The results are expressed as the plasma titer giving 50% reduction in infectivity relative to a positive control of virus infected cells.

Histologic Sampling and Immunohistochemical Staining of Resected Tumor Specimens

Resected tumor specimens were serially sliced and the whole tumor was processed into paraffin blocks. This resulted in a median of 24 blocks per case (range, 9-80 blocks). Slices were photographed and blocks were numbered serially to allow reconstruction of their location within the tumor mass. The samples included surrounding non-neoplastic liver tissue. All blocks thus obtained were stained immunohistochemically using sheep polyclonal antiserum raised against recombinant NTR. Immunostaining was performed on a complete set of sections from two levels 200 μm apart, through each tumor plus associated nontumor tissue. The sheep anti-NTR primary antibody was used at a dilution of 1:8,000 for 1 hour at room temperature and visualized by a Sheep Vectastain Elite ABC kit and Vector AEC chromagen (Vector Laboratories Ltd, Peterborough, UK). The proportion of tumor cells, normal hepatocytes, lymphocytes, and stromal cells expressing NTR was assessed and the percentage of sections with positive staining per tumor was determined. For adenovirus receptor evaluation, a rabbit polyclonal antiserum to the Coxsackie and Adenovirus receptor (CAR) was used (provided by Dr G.E. Blair, University of Leeds, Leeds, UK).[16] Staining was carried out using an agitated low temperature epitope retrieval technique with the primary antibody at a concentration of 1 in 200. This was visual-

Table 1. CTL102 Dose Escalation

Dose Level (virus particles)	No. of Patients	Colorectal/Hepatoma	Nonresectable	Toxicity (grade 1 fever)
1×10^8	3	3/0	0	0
1×10^9	3	3/0	1 (biopsied)	0
1×10^{10}	4	4/0	1	1
1×10^{11}	5	3/1 (+1 benign lesion)	1	2
5×10^{11}	3	3/0	2	1

ized with the ChemMate EnVision Detection Kit (Dako Ltd, Ely, UK) and Vector NovaRed chromagen (Vector Laboratories Ltd, Peterborough, UK).

RESULTS

Patients

Eighteen patients aged between 51 and 79 years (median, 65 years) were treated; 11 male, and seven female. Sixteen patients had hepatic metastatic colorectal cancer; one patient had hepatocellular carcinoma on a background of primary biliary cirrhosis; one patient with a history of Dukes' C colorectal cancer with hepatic lesions identified by ultrasonography and computed tomography was subsequently found to have benign hemangioma at laparotomy. Following CTL102 administration, surgical resection of the tumor was undertaken 3 to 8 days later. In five patients, extra-hepatic disease was found at laparotomy, rendering hepatic resection futile. In this event, a further patient was recruited to allow analysis of transgene expression in at least three patients per dose level, unless a surgical biopsy was obtained. Thus, overall, 18 patients were assessable for toxicity, and in 13 of 18 patients, transgene expression could be studied. Virus dose ranged from 1×10^8 particles to 5×10^{11} particles (Table 1).

Toxicity

No dose-limiting toxicity has been observed up to and including the maximum dose of 5×10^{11} virus particles. Some patients experienced mild local pain at the injection site, which was relieved by simple analgesia. Four patients were noted to have asymptomatic grade 1 pyrexia ($\leq$ 38.5°C) between 4 and 8 hours post-treatment, although no change in inflammatory markers (erythrocyte sedimentation rate, c-reactive protein, interleukin-6) was observed in these or any other patients. No hepatic, gastrointestinal, or hematologic toxicity has been observed at any dose level.

Virus Shedding

ELISA for adenovirus proteins in plasma, throat swab, urine, and stool found no detectable virus at any dose at 24 hours following intratumoral injection of CTL102 (data not shown).

Virus Kinetics

Quantitative PCR using a CTL102-specific primer and probe combination demonstrated that vector DNA was detectable in the blood of most patients within 30 minutes following intratumoral injection, declining to undetectable levels by 8 to 24 hours for most patients (Fig 1). No clear correlation between dose of CTL102 and level and duration of vector DNA detectable in blood was observed, although there appears to be a general trend towards more circulating DNA and longer clearance time with higher doses.

Immune Responses to CTL102

Before injection of CTL102, most patients had detectable antibodies to adenovirus as measured in a group-specific ELISA, detecting total anti-adenovirus Ig. All patients showed an increase in titer between 7 and 14 days post-treatment, reaching a plateau by 14 to 32 days (Fig 2A). ELISA tests for IgM, IgA, and IgG showed a similar pattern (not shown). There was no clear seroconversion from an IgM response to IgG in any patient.

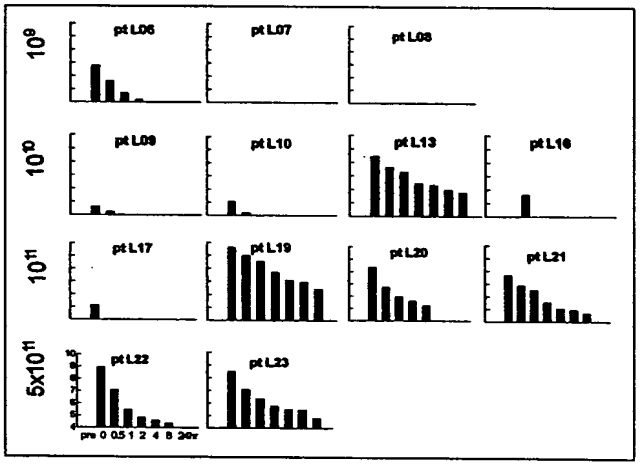

Fig 1. Quantitative PCR for CTL102 in peripheral circulation following direct intratumoral injection. Total DNA was extracted from 200 μL aliquots of blood collected before, immediately at the end of injection, and at intervals thereafter, and used in Taqman QPCR for CTL102. The axes in all the subpanels are the same: y-axis, CTL102 copy number ($\log_{10}$); x-axis, time (hours) post CTL102 injection.

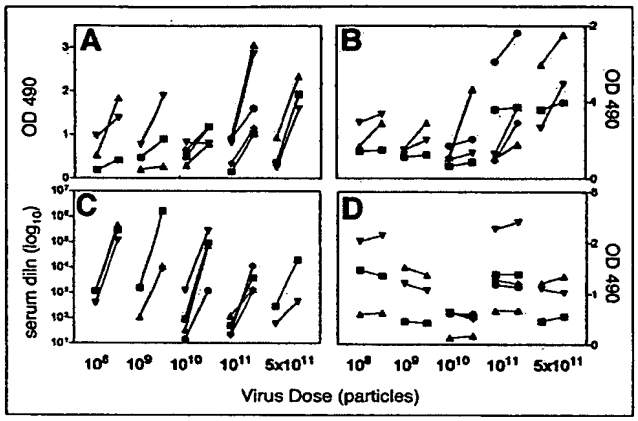

Fig 2. Antibody responses to CTL102. Plasma samples were taken within 2 weeks prior to treatment and at weekly, then monthly, intervals after treatment, and tested by enzyme-linked immunosorbent assay for antibodies recognizing (A) adenovirus; (B) nitroreductase; (C) for type specific neutralization to Ad5; and (D) influenza A. The pretreatment and peak response titers are shown. The symbols correspond to first, ▲; second, ▼; third ■; fourth ●; and fifth ◆ patient in each cohort.

The assay for antibodies to NTR revealed that most patients had a detectable, albeit low, response that was substantially increased after exposure to CTL102 (Fig 2B). Tests of normal volunteers showed that low levels of antibodies to NTR are present in a majority of individuals (12 of 13 tested). To provide evidence that the rise in antibody titer was not simply a reflection of a nonspecific rise in antibodies following surgery, patients were tested for antibodies to influenza A, and minimal changes were recorded (Fig 2D).

Neutralization tests using Ad5 β-galactosidase showed that most patients had specific activity against Ad5 before receiving CTL102. All patients showed enhanced neutralization at 14 to 28 days following treatment (Fig 2C). There was no correlation between CTL102 dose or level of virus DNA in blood and the extent of antibody responses, or between the level of pre-existing antibody and the magnitude of the response.

Immunohistochemical Analysis of Resected Tumors

Immunohistochemical staining for NTR in resected tumors confirmed transgene expression even at the lowest dose of 1×10^8 virus particles (Fig 3). Staining was both nuclear and cytoplasmic in location. NTR expression was focal in distribution, especially at lower dose levels, where staining was present in less than 1% of tumor cells per section. A dose-related increase in NTR expression was seen with each increment, with an increase in both the number of NTR positive cells per slide (Fig 3) and the number of slides with any NTR staining per tumor (Fig 4). At the dose 1×10^{11} virus particles, strong NTR expression was seen in approximately 50% of slides (78 of 146) from sectioned tumor and up to 20% of cells per slide were NTR-positive. NTR staining was present in tumor cells and also in other cell types within the tumor. Morphologically, these cells resembled lymphocytes, macrophages, and fibroblasts. Dual immunostaining with CD45, CD68, vimentin, and CD31 indicated that the majority of nontumor cells expressing NTR were CD68-positive macrophages (data not shown). At the highest dose of 5×10^{11} virus particles, some NTR expression was also observed in adjacent normal liver (Fig 3F). In the case of hepatocellular carcinoma, NTR was expressed more extensively in tumor cells; focally up to 50% of cells per field were staining positively (Fig 3E).

Immunohistochemical staining for the CAR demonstrated high expression throughout the tumor such that uptake of virus was unlikely to be limited by the lack of the primary virus receptor (Figs 3G and H).

DISCUSSION

This study provides the first description of the extent and cellular distribution of transgene expression achieved after direct intratumoral injection of a replication-defective adenovirus vector to patients, followed by complete tumor resection.

The primary objective of the study was to determine the safety and tolerability of the adenovirus vector CTL102. The protocol employed a novel design to allow measurement of NTR expression in resected tumors in order to assess the dose of CTL102 required to transduce a proportion of cells sufficient for significant prodrug activation. Additional objectives were to measure the kinetics of CTL102 distribution following intratumoral injection, and to assess host immune responses to the vector and transgene.

Up to the maximum dose of 5×10^{11} virus particles, no dose-limiting toxicity has been observed. The only adverse events were pain at the site of injection and asymptomatic low-grade pyrexia in some patients, occurring in a dose-independent manner. No change in the biochemical estimates of liver function was seen, suggesting that the doses and delivery route used may minimize potential adenovirus-mediated liver toxicity.

After intratumoral administration of CTL102, virus DNA was detectable in the blood of patients as soon as 15 to 30 minutes postinjection, indicating some vector dissemination from the injection site. In most cases, CTL102 DNA was undetectable 8 to 24 hours later.

CTL102 injection provoked IgG, IgM, and IgA anti-adenovirus antibody responses. These antibodies were able to neutralize Ad5 in vitro. Such antibody responses may provide a level of safety against systemic exposure to the virus. However, they may also present a potential obstacle to efficacy of repeated systemic administration of the vector.

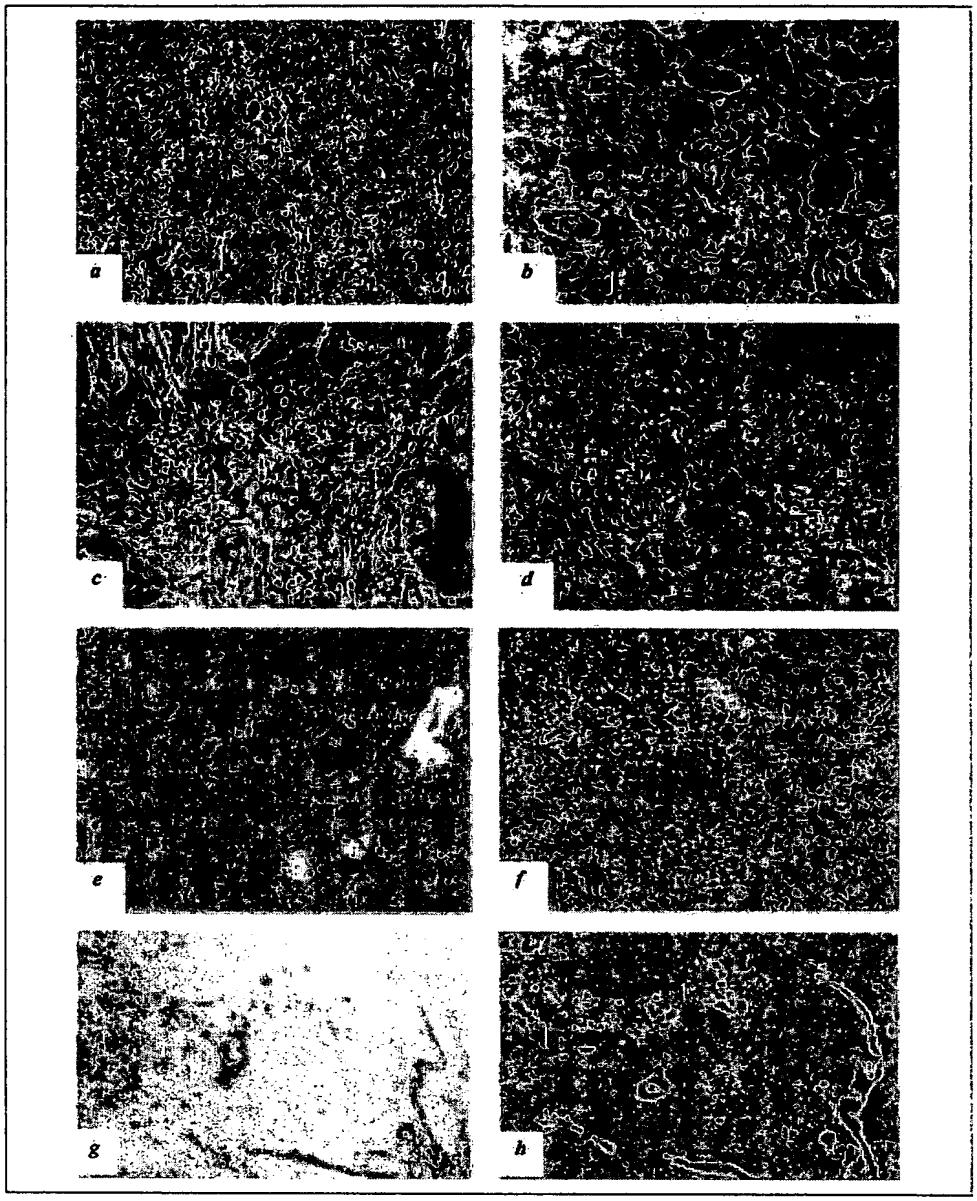

Fig 3. Immunohistochemical staining of tumor sections with anti-nitroreductase (NTR) antibody. (A) 10^8 virus particles; (B) 10^9 virus particles; (C) 10^{10} virus particles; (D-E) 10^{11} virus particles; (F) 5×10^{11} virus particles (× 200 magnification); (G-H) correlation between distribution of primary adenovirus receptor and NTR expression in tumor sections. Tumor was injected with CTL102 (1×10^{10} virus particles) prior to resection. (A-D, F) Colorectal metastases; (E) hepatocellular carcinoma. Serially cut sections were immunostained for (G) NTR and (H) Coxsackie and Adenovirus receptor.

This study has demonstrated the successful application of the CTL102 vector to express nitroreductase in tumor cells when administered by direct intratumoral injection. Further, we have shown that the level of prodrug-activating enzyme in tumors, likely to be a limiting component of VDEPT, increases in proportion to CTL102 dose. NTR was not confined to tumor cells but was also seen in tumor-associated macrophages. However, this could still catalyze activation of the prodrug to facilitate bystander tumor cell killing. Immunohistochemical staining for the CAR receptor and NTR indicates colocalization. The extent of CAR staining within a tumor exceeded NTR positivity, indicating that saturation of CAR should not be a limiting step in the development of this system. In view of this and the lack of toxicity at the current maximal dose of 5×10^{11} particles, it will be feasible to continue dose escalation in order to define a maximum-tolerated dose.

At the dose level of 1×10^{11} particles, we have observed NTR expression in more than 50% of pathologic sections comprising the tumor. Based on preclinical data, this level of enzyme expression should be sufficient to catalyze significant activation of the prodrug. In a

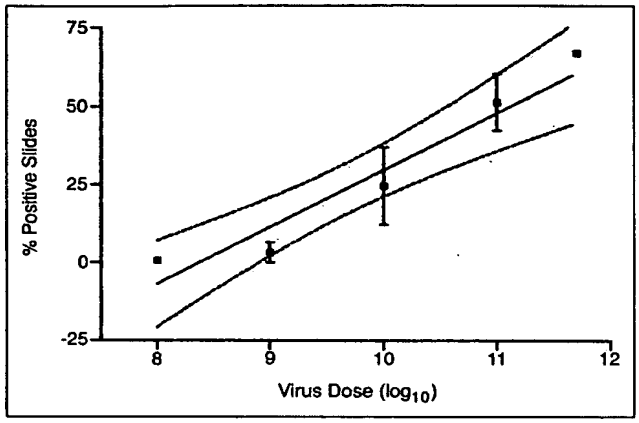

Fig 4. Quantitation of nitroreductase staining. The number of nitroreductase positive slides expressed as a percentage of the total number of slides for each tumor was analyzed by linear regression. The results are the mean of three tumors for each dose (except the highest dose). The broken lines indicate 95% confidence limits.

previous study, we have established that a safe dose of CB1954 can achieve plasma concentrations likely to be sufficient to generate a clinically effective level of the activated species in the presence of NTR.[12] On the basis of these data, we are now proceeding with the next stage in the development of this VDEPT approach, using the CTL102 vector administered by intratumoral injection followed 48 hours later by IV administration of CB1954 to assess the safety and tolerability of the combination, and to look for evidence of efficacy by inoculating individual tumor nodules of patients with nonresectable primary or secondary liver cancer.

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Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Acted as a consultant within the last 2 years: Vivien Mautner, ML Laboratories; David J. Kerr, ML Laboratories; Lawrence S. Young, ML Laboratories; Stefan Hubscher, ML Laboratories. Received more than $2,000 a year from a company for either of the last 2 years: Vivien Mautner, ML Laboratories; David J. Kerr, ML Laboratories; Lawrence S. Young, ML Laboratories; Stefan Hubscher, ML Laboratories.

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